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Plasma/Serum Circulating DNA Purification Mini Kit Dx (Slurry Format)
Product Insert

REF **Dx50600** CE IVD PIDx50600-1

Intended Use

Norgen's Plasma/Serum Circulating DNA Purification Mini Kit Dx (Slurry Format) provides a fast, reliable and simple procedure for isolating circulating DNA from plasma/serum for subsequent *in vitro* diagnostic use. DNA can be isolated from either fresh or frozen samples using this kit. Purification is based on the use of Norgen's proprietary resin as the separation matrix, and the kit is able to isolate all sizes of circulating DNA.

This kit is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of DNA followed by signal detection or amplification. Any diagnostic results generated using the DNA isolated with Plasma/Serum Circulating DNA Purification Mini Kit Dx (Slurry Format) in conjunction with an *in vitro* diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

To minimize irregularities in diagnostic results, suitable controls for downstream applications should be used.

Norgen's Plasma/Serum Circulating DNA Purification Mini Kit Dx (Slurry Format) is intended for use by professional users such as technicians, physicians and biologists experienced and trained in molecular biological techniques including DNA isolation.

Norgen's Plasma/Serum Circulating DNA Purification Mini Kit Dx (Slurry Format) does not provide a diagnostic result. It is the sole responsibility of the user to use and validate the kit in conjunction with a downstream *in vitro* diagnostic assay.

Kit Components

Component	Product #Dx50600 (50 samples)
Lysis Solution	65 mL
Binding Solution 1	12 mL
Binding Solution 2	10 mL
Wash Solution	2 x 22 mL
Elution Buffer	6 mL
Mini Filter Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
Product Insert	1

Label Legend

Do not reuse	Use by	Batch Code	Catalogue Number	Contains sufficient for <n> tests	Manu-facturer	In Vitro Diagnostic Medical Device	Consult instructions for use	Temper-ature limitation

Made in Canada
Norgen Biotek

Advantages

- CE-IVD marked in accordance with EU Directive 98/79/EC
- Fits into *in vitro* diagnostic workflows
- Fast and easy processing using a rapid spin-column format
- Isolate high quality genomic DNA, free from RNA contamination

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. All solutions and plastics can be used until the expiration date specified on their labels.

Precautions

Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

The **Lysis Solution, Binding Solution 1** and **Binding Solution 2** contain guanidine hydrochloride, and should be handled with care. Guanidinium salts forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

Plasma or Serum of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with plasma or serum.

Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- Micropipettors
- 96 – 100% ethanol
- 60°C incubator
- 2 mL tubes

Procedure

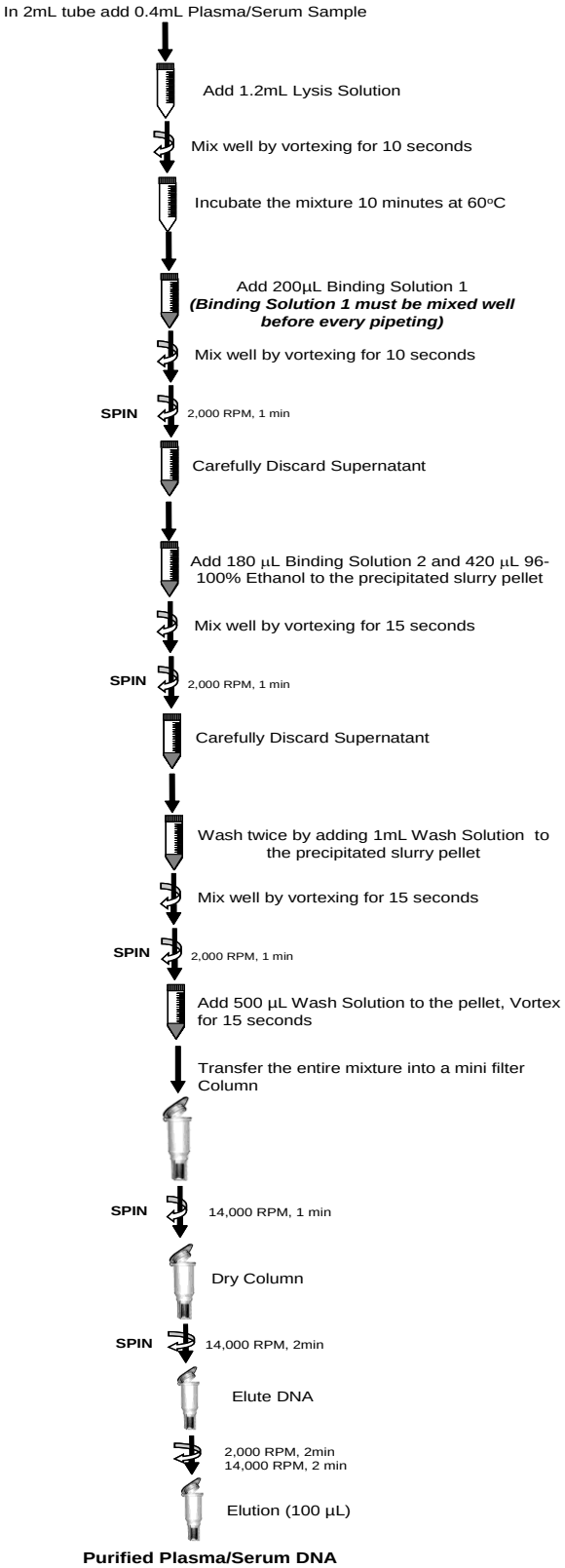
All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})\,(r)}}$$

where *RCF* = required gravitational acceleration (relative centrifugal force in units of g); *r* = radius of the rotor in cm; and *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force

Flow Chart

Procedure for Purifying Plasma/Serum DNA using Norgen's Plasma/Serum Circulating DNA Purification Mini Kit Dx (Slurry Format)


In 2mL tube add 0.4mL Plasma/Serum Sample
Add 1.2mL Lysis Solution
Mix well by vortexing for 10 seconds
Incubate the mixture 10 minutes at 60°C
Add 200µL Binding Solution 1
(Binding Solution 1 must be mixed well before every pipeting)
Mix well by vortexing for 10 seconds
SPIN
2,000 RPM, 1 min
Carefully Discard Supernatant
Add 180 µL Binding Solution 2 and 420 µL 96-100% Ethanol to the precipitated slurry pellet
Mix well by vortexing for 15 seconds
SPIN
2,000 RPM, 1 min
Carefully Discard Supernatant
Wash twice by adding 1mL Wash Solution to the precipitated slurry pellet
Mix well by vortexing for 15 seconds
SPIN
2,000 RPM, 1 min
Add 500 µL Wash Solution to the pellet, Vortex for 15 seconds
Transfer the entire mixture into a mini filter Column
SPIN
14,000 RPM, 1 min
Dry Column
SPIN
14,000 RPM, 2min
Elute DNA
2,000 RPM, 2min
14,000 RPM, 2 min
Elution (100 µL)
Purified Plasma/Serum DNA

Notes prior to use:

- Ensure that all solutions are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.
- All centrifugation steps are performed at room temperature.
- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- Preheat an incubator or heating block to 60°C.
- Prepare a working concentration of the **Wash Solution** by adding 50 mL of 96-100% ethanol (provided by the user) to the supplied bottles containing the concentrated Wash Solution. This will give a final volume of 72 mL. The labels on the bottles have a box that may be checked to indicate that the ethanol has been added.
- Ensure that samples have not undergone more than one freeze-thaw cycle, as this may lead to DNA degradation.
- ***Binding Solution 1 contains resin and must be mixed well before every pipetting***.
- ***This kit is suitable for the isolation of DNA from fresh or frozen serum or plasma prepared from blood collected on either Heparin, EDTA or citrate.***

Detailed Procedure

1. Add 400 µL of Plasma/Serum sample to a 2 mL tube (not provided).
2. To each 400 µL Plasma/Serum sample add 1.2 mL of **Lysis Solution**. Mix well by vortexing for 15 seconds.
3. Incubate the mixture from **Step 2** for 10 minutes at 60^{o}C
4. After incubation add 200 µL of **Binding Solution 1** and mix well by vortexing for 15 seconds. ***(Note*: *Binding Solution 1 contains resin and must be mixed well before every pipeting)***
5. Centrifuge the mixture from **Step 4** for **1 minute at 200 x g (~2,000 RPM)**, then carefully decant the supernatant in order to ensure that the slurry pellet is not dislodged.
6. To the slurry pellet from **Step 5** add 180 µL from **Binding Solution 2** followed by the addition of 420 µL of **96-100% Ethanol** (provided by the user). Mix well by vortexing for 15 seconds.
7. Centrifuge the mixture from **Step 6** for **1 minute at 200 x g (~2,000 RPM)**, then carefully decant the supernatant in order to ensure that the slurry pellet is not dislodged.
8. To the slurry pellet from **Step 7** add 1 mL **Wash Solution**, mix well by vortexing for 15 seconds and then centrifuge for **1 minute at 200 x g (~2,000 RPM)**. Carefully decant the supernatant in order to ensure that the slurry pellet is not dislodged.
9. Repeat **Step 8** to wash the slurry pellet for a second time.
10. To the slurry pellet from **Step 9** add 500 µL **Wash Solution,** Mix well by vortexing for 15 seconds.
11. Transfer the entire mixture from **Step 10** into a Mini Filter Spin column. Centrifuge for **1 minute at 10,000 x g (~14,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube.
12. Spin the column, empty, for **2 minute at 10,000 x g (~14,000 RPM)**. Discard the collection tube.
13. Transfer the spin column to a fresh 1.7 mL Elution tube. Apply 100 µL of **Elution Buffer** to the column and centrifuge for **2 minutes at 200 x g (~2,000 RPM),** followed by **2 minute at 10,000 x g (~14,000 RPM)**.

Frequently Asked Questions

1. What If a variable speed centrifuge is not available?
- A fixed speed centrifuge can be used, however reduced yields may be observed.

2. What will happen if my centrifugation speed varied from the recommended speed?
- This may lead to the degradation of the isolated DNA or reduction in the total DNA yields.

3. At what temperature should I centrifuge my samples?
- All centrifugation steps are performed at room temperature. Centrifugation at 4°C will not adversely affect kit performance.

4. What If I added more or less of the specified reagents' volume?
- Adding less volume may reduce both the quality and the quantity of the purified DNA. Adding more may not affect the nucleic acid yields EXCEPT if more Elution Buffer was added. Eluting your DNA in higher volumes will result in diluting your nucleic acids.

5. What If my incubation varied from the 10 minutes specified in the product manual?
- Less than 10 minutes will result in a lower DNA yields and poor DNA quality. More than 10 minutes may not affect your DNA yields.

6. What If I forgot to do a dry spin after my second wash?
- Your elution will be contaminated with the Wash Solution. This may dilute the nucleic acid yield in your first elution and may interfere with your down stream applications.

7. Can I elute in a smaller volume?
- Yes, you can. The minimum elution volume that can applied to the column is 50 μL. After eluting with 50 μL, re-load the eluted 50 μL back to the column and re-elute with the same 50 μL. This will make the final elution volume 50 μL.

8. Why do my samples show very low DNA yield?
- Plasma/Serum samples contain very little DNA. This varies from individual to individual based on numerous variables.

9. Why my isolated nucleic acids do not perform well in downstream applications?
- If a different Elution Buffer was used other than the one provided in the kit, the buffer should be checked for any components that may interfere with the application. Common components that are known to interfere are high salts (including EDTA), detergents and other denaturants. Check the compatibility of your elution buffer with the intended use.

10. Do I need to do an RNase treatment for my DNA Elution?
- Unlike other kits, Norgen's Plasma/Serum Circulating DNA Purification Mini Kit (Slurry Format) doesn't co-purify plasma/serum circulating RNA along with circulating DNA, therefore an RNase step is not required.

11. Why do the A260:280 ratio and the A260:230 ratio of the purified DNA are low?
- Most of the free-circulating Plasma/Serum DNA is present in short fragment. This low A260:280 ratio and the low A260:230 ratio will not affect any downstream application

Technical Support

Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

Product Use Restriction

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The respective user is liable for any and all damages resulting from application of Norgen's Plasma/Serum Circulating DNA Purification Mini Kit Dx (Slurry Format) for use deviating from the intended use as specified in the user manual.

All products sold by Norgen Biotek are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately. The kit contents are for laboratory use only, and they must be stored in the laboratory and must not be used for purposes other than intended. The kit contents are unfit for consumption.

Authorized Representative

EC REP

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